

# The impact of preanalytical factors on glucose concentration measurement



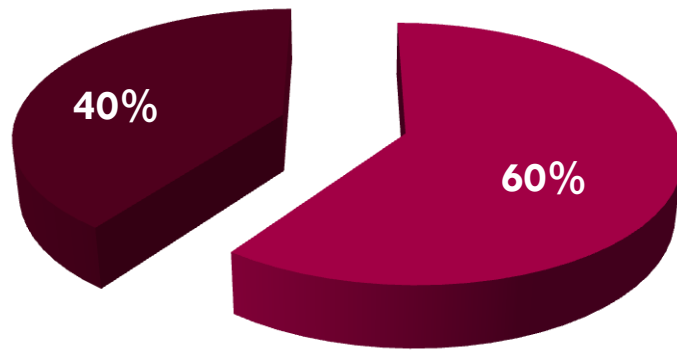
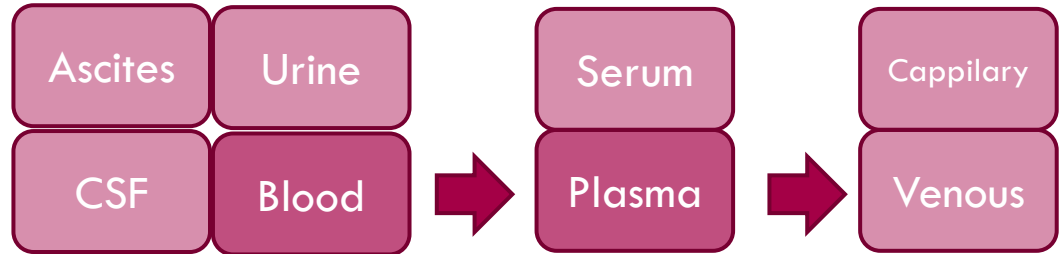
14th EFLM Continuing Postgraduate Course in Clinical Chemistry and Laboratory Medicine  
New trends in laboratory diagnosis and management of diabetes mellitus:  
Diabetes mellitus revisited 14 years after the first Dubrovnik course  
October 25-26, 2014, Dubrovnik, Croatia

Nora Nikolac, PhD

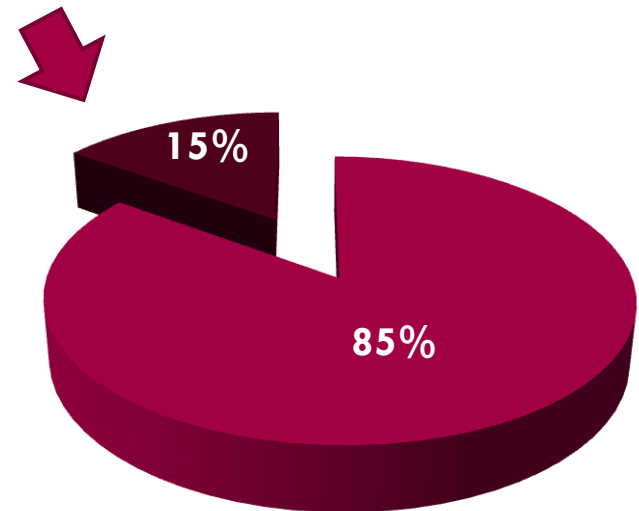
University Department of Chemistry, Medical School  
University Hospital Sestre Milosrdnice, Zagreb, Croatia

# Glucose concentration measurement

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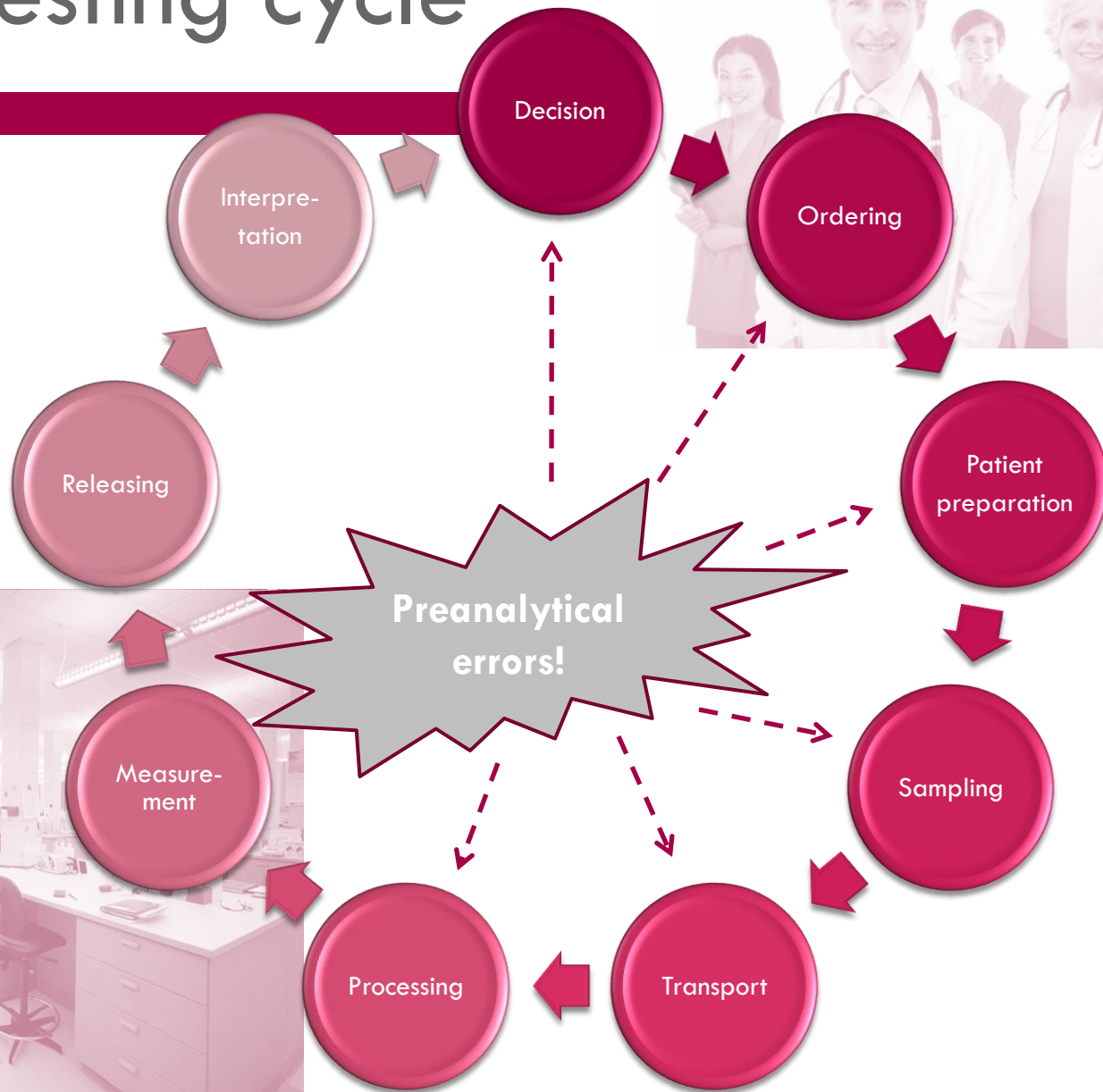
- Samples without glucose
- Samples with glucose



- Other biochemistry tests
- Glucose

# Total testing cycle

3



# Sources of preanalytical errors

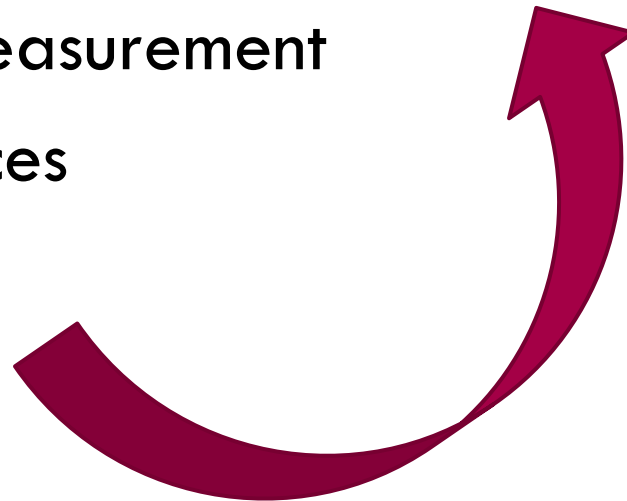
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## Variability

- ❑ Patient preparation
- ❑ Sample type
- ❑ Type of container
- ❑ Time of measurement
- ❑ Interferences

## Diagnostic errors

- ❑ Delayed diagnosis
- ❑ Misdiagnosis
- ❑ Wrong diagnosis



# 1. Patient preparation

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## Fasting glucose

- Fasting time?
- Definition of fasting?

## Postprandial glucose

- Type of meal?
- Therapy (medications)

## Oral glucose tolerance test

- Adherence to instructions
- Gestational DM

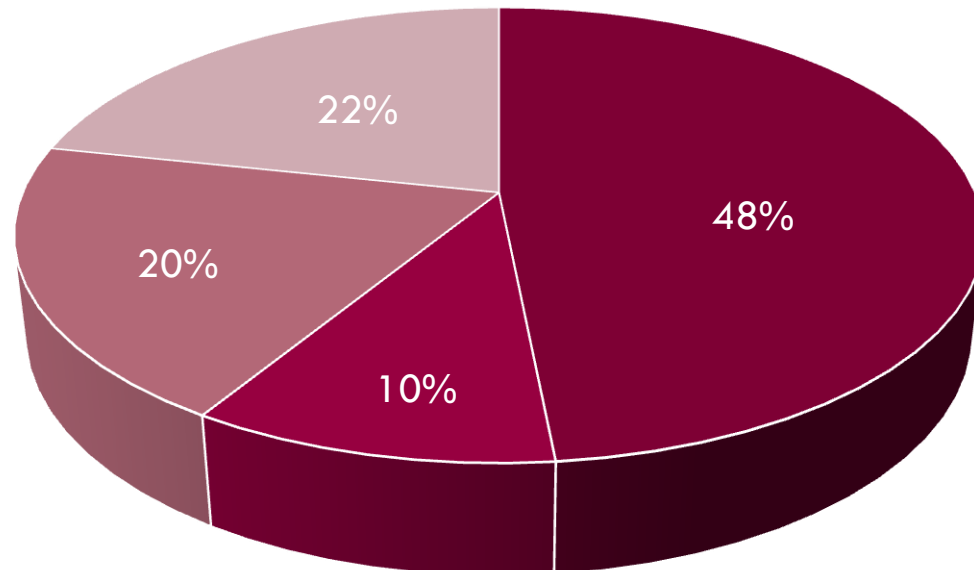
# Croatian survey

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- **CSMBLM – WG** for patient preparation
- March 2014
- Online survey on practices for patient preparation
- Heads of the laboratories
- Response rate:  $118/206 = 57\%$



**Question 13:** According to your instructions for patient preparation, what is the required fasting time for glucose concentration measurement:



- A) at least 8 hours
- B) at least 10 hours
- C) at least 12 hours
- D) number of hours not specified

# Definition of fasting?

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## Original papers

### Are patients well informed about the fasting requirements for laboratory blood testing?

Sanja Kackov<sup>1\*</sup>, Ana-Maria Simundic<sup>2</sup>, Ani Gatti-Drnic<sup>3</sup>

<sup>1</sup>Medical biochemistry laboratory, Policlinic Bonifarm, Zagreb, Croatia

<sup>2</sup>University Department of Chemistry, Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia

<sup>3</sup>Medical biochemistry laboratory, Public Health Centre Zagreb-Centar, Zagreb, Croatia

\*Corresponding author: sanjakackov@hotmail.com

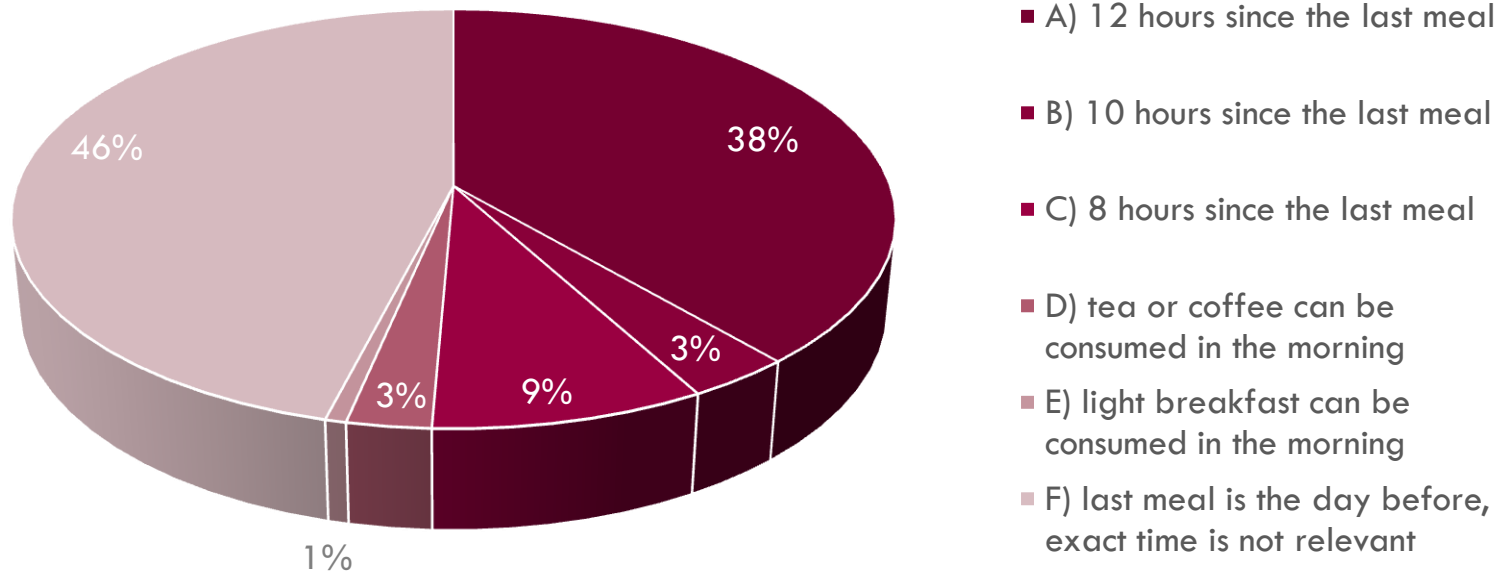
*Biochem Med 2013;23(3):326-31.*

- Survey on outpatients in the primary care laboratory
- N = 150



Kackov S, Simundic AM, Gatti-Drnic A. Are patients well informed about the fasting requirements for laboratory blood testing? *Biochem Med* 2013;23(3):326-31.

### What does the fasting state mean?



# Harmonization and education



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- Patients are not informed
- Heterogeneity of instructions
- Revision of existing guidelines
- Education of patients and laboratory staff



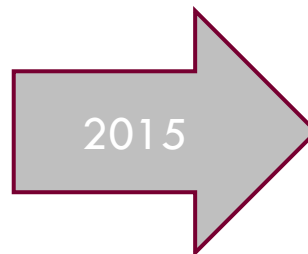
Hrvatsko društvo za medicinsku  
biokemiju i laboratorijsku medicinu

Croatian society of medical  
biochemistry and laboratory medicine

CSMBLM

WG for

patient preparation



**National  
recommendation on  
patient preparation**

## 2. Sample type

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**Plasma** sample



- Lower water content
- Consumption of glucose during clotting



**Serum** sample

## 2. Sample type

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Venous sample

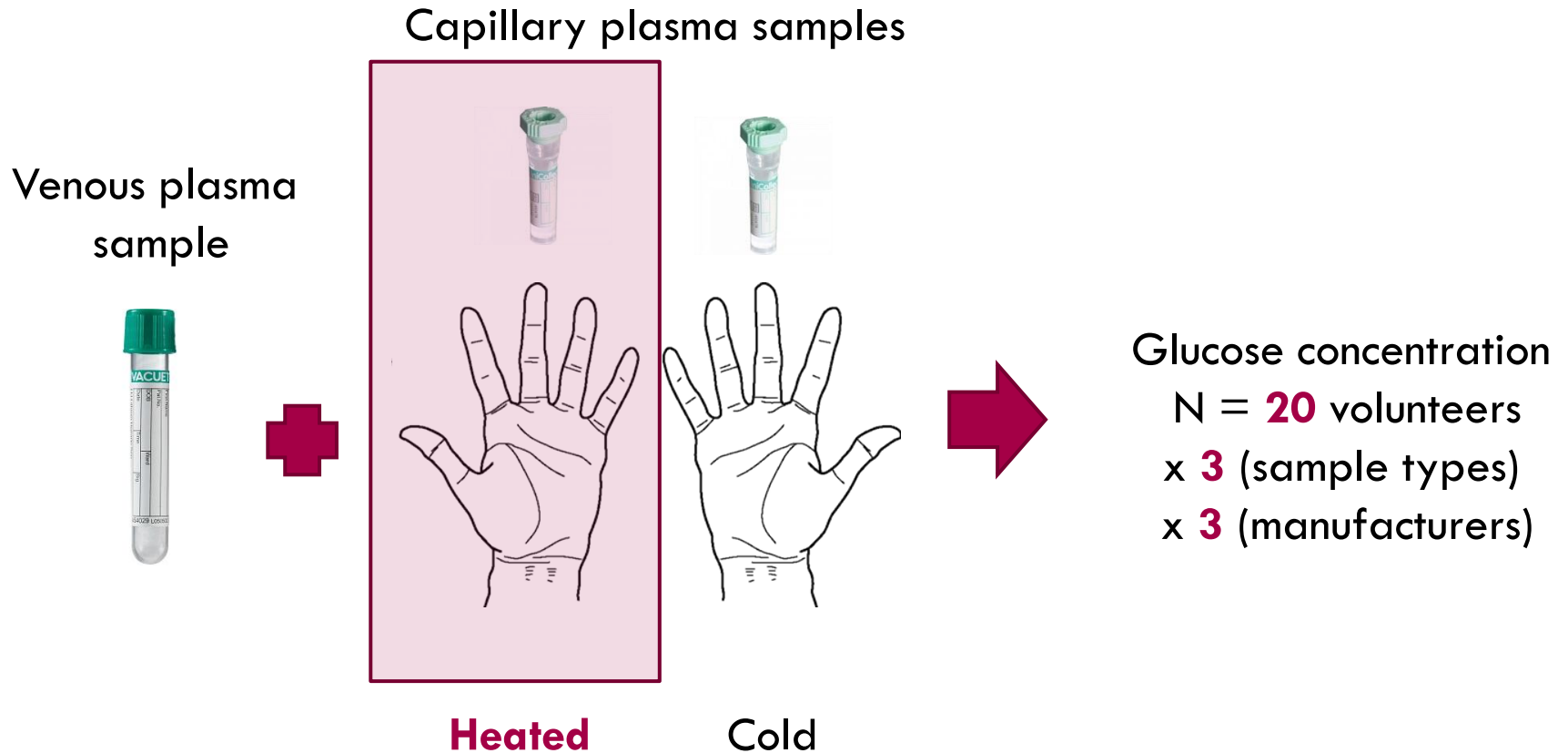


Capillary sample

- Rate of the glucose consumption in the tissues

# Quality of the capillary sample?

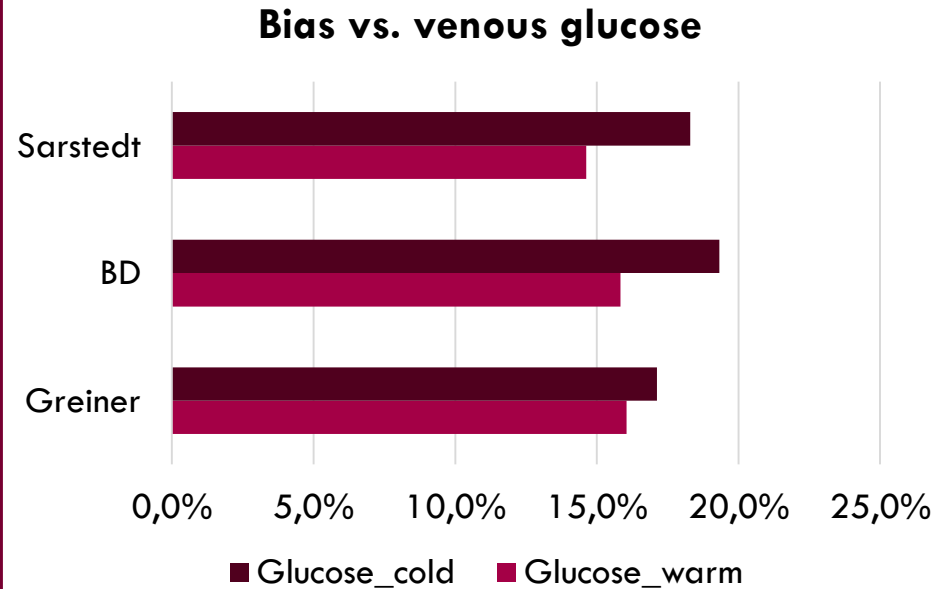
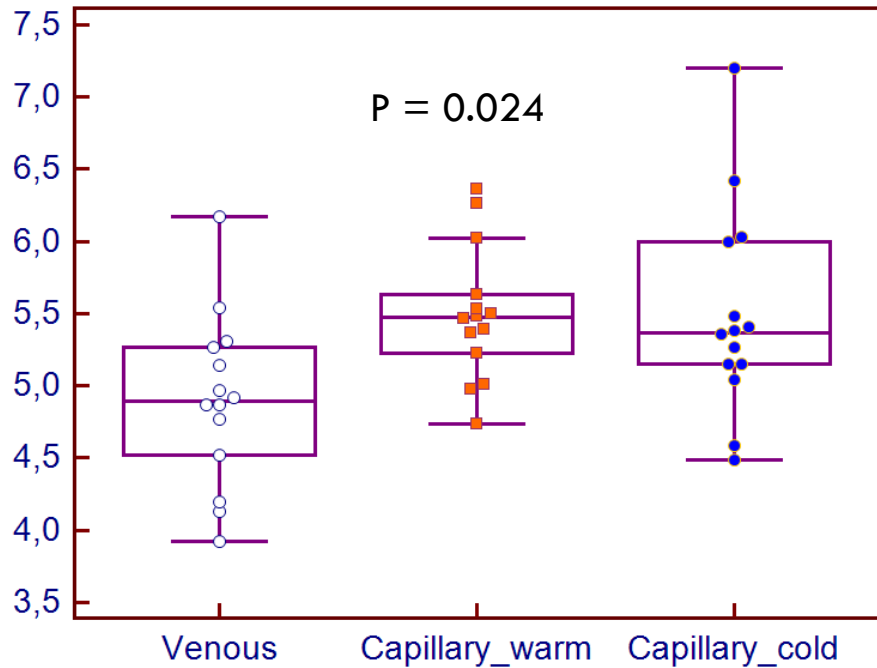
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*Simundic AM, Nikolac N, et al. Capillary sample quality: verification of three different lancets for capillary blood sampling; **Publication in process.***

*Simundic AM, Nikolac N, et al. Capillary sample quality: verification of three different lancets for capillary blood sampling; Publication in process.*

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## 2. Sample type

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- ❑ Serum/plasma and venous/capillary samples can not be used interchangeably
- ❑ Glucose measurement should always be performed in the same sample type



# 3. Type of container/ Time of measurement



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- Metabolic processes continue *in vitro* (glucose, ammonia, lactate)



icy water  
slurry



rapid  
centrifugation  
(30 min)



separation from  
the cells



special additives  
to block the process

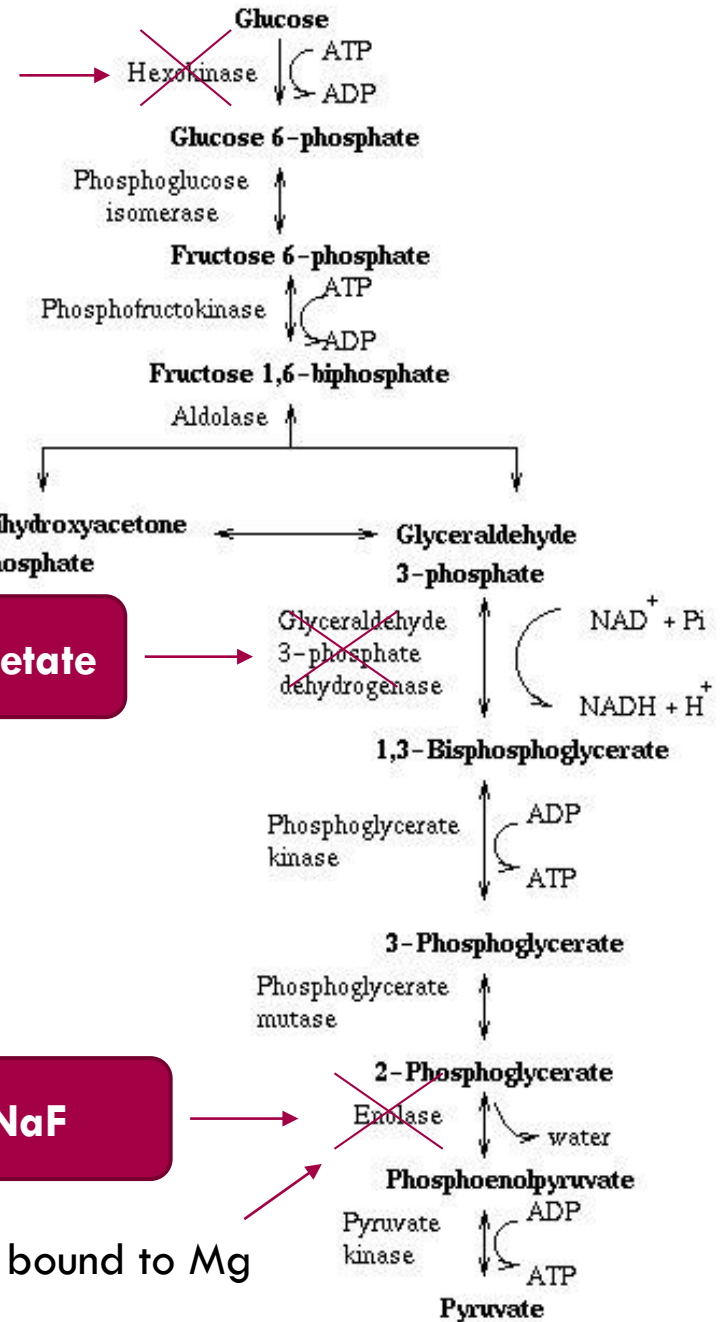




# Glycolysis

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**Mannose**



F + inorganic phosphates  $\rightarrow$  bound to Mg

# Is NaF efficient?

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> ~5%  
(~0.4 mmol/L)

> ~5%

Immediate centrifugation and separation from cells is superior to NaF!



NaF has up to 3 hours delay in glycolysis inhibition!

*Shi RZ, et al. Rapid blood separation is superior to fluoride for preventing in vitro reductions in measured blood glucose concentration. J Clin Pathol 2009;62:752-3.*

*Waring WS, et al. Glycolysis inhibitors negatively bias blood glucose measurements: potential impact on the reported prevalence of diabetes mellitus. J Clin Pathol 2007;60:820-3.*

# Problem with NaF?

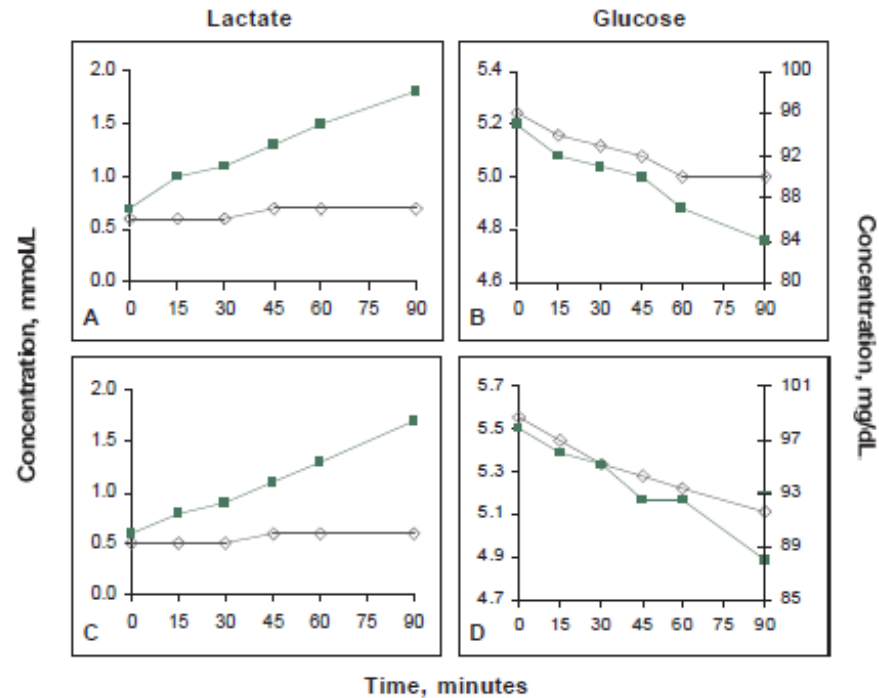
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**Fluoride** waiting for 30-90 minutes?

# NaF mechanism

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**Fig. 1. Changes in lactate and glucose concentrations over time.**

Specimens of whole blood were collected and stored at room temperature in Vacutainer tubes (BD) containing either lithium heparin (filled squares) or fluoride/oxalate (open diamonds). Over a 90-min period, samples were removed from each tube and centrifuged to obtain plasma for measurements of lactate and glucose. Panels A and B, volunteer 1; panels C and D, volunteer 2.

Mikesh LM, Bruns DE. Stabilization of glucose in blood specimens: mechanism of delay in fluoride inhibition of glycolysis *Clin Chem* 2008;54:930-2.

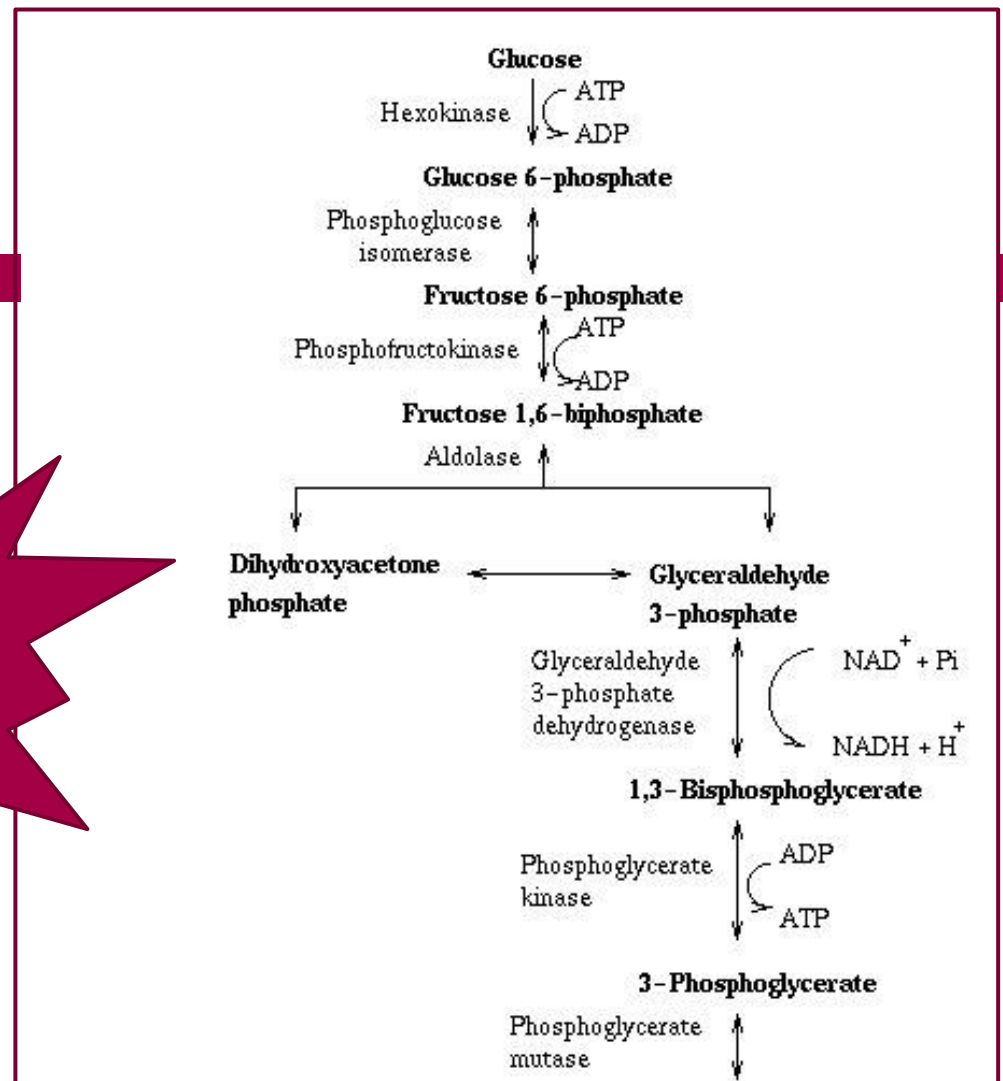
**NaF blocks production of lactate immediately!**

# Glycolysis

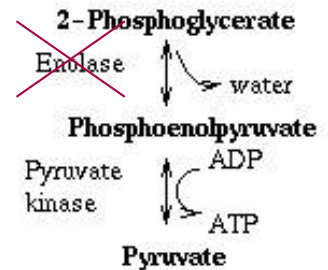
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Phosphorilation of sugars occurs until ATP is exhausted (60-90 minutes)

↓ 5-7% per hour

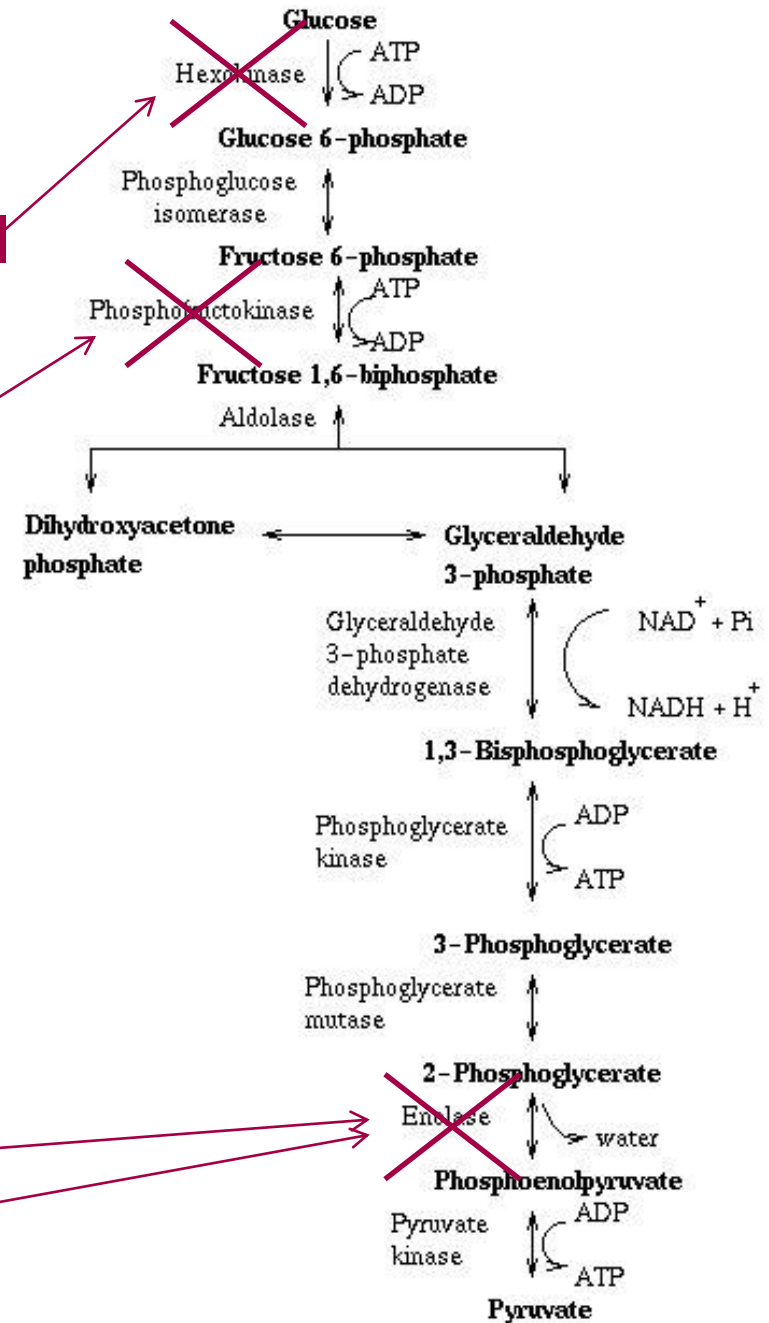
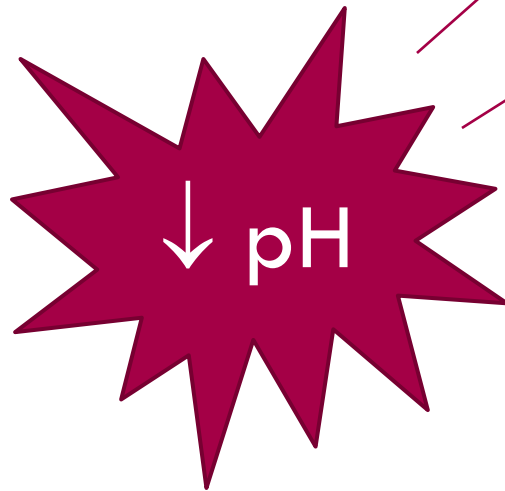


NaF



# (Not so) New inhibitors?

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Patent in 1986 by Terumo corporation:

- ❑ Citric acid, trisodium citrate pH 5.9
- ❑ Disodium EDTA (chelate Mg)
- ❑ NaF (prolonged inhibition)

# Glucose concentration in the new tubes?

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Clinical Chemistry 55:5  
1019-1021 (2009)

## Acidification of Blood Is Superior to Sodium Fluoride Alone as an Inhibitor of Glycolysis

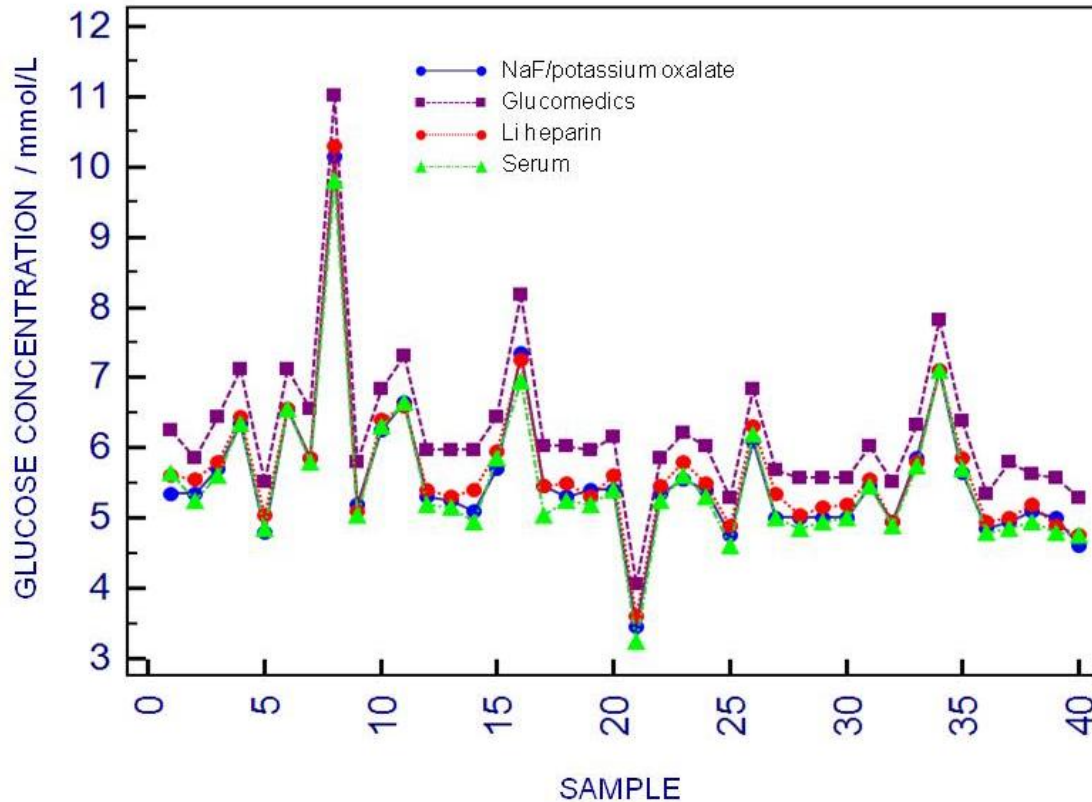
Raymond Gambino,<sup>1\*</sup> Janet Piscitelli,<sup>2</sup> Tomy A. Ackattupathi,<sup>2</sup> Judy L. Theriault,<sup>3</sup> Reynaldo D. Andrin,<sup>3</sup> Michael L. Sanfilippo,<sup>4</sup> and Monina Etlenne<sup>4</sup>  
Quest Diagnostics, <sup>1</sup> Deerfield Beach, FL, <sup>2</sup> Teterboro, NJ, <sup>3</sup> West Hills, CA, and <sup>4</sup> Miramar, FL; \* address correspondence to this author at: Quest Diagnostics, 1300 East Newport Center Dr., Deerfield Beach, FL 33442-7727. E-mail Raymond.X.Gambino@questdiagnostics.com.

	Loss of glucose 2 h	Loss of glucose 24 h
Citrate buffer	↓ 0.3%	↓ 1.2%
NaF tube	↓ 4.6%	↓ 7.0%

**Minimal loss of glucose in the first 2 hours, stable up to 24 hours.**

*Juricic G, Milevoj Kopcinovic L, Saracevic A, Bakliza A, Simundic AM. Do citrate buffer tubes introduce a new era of glucose measurement? **Publication in process.***

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**Baseline:**

Citrate buffer  
(Glucomedics)



Heparin  
plasma



NaF/KOx



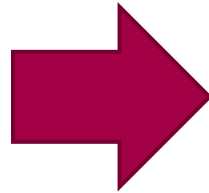
Serum





# Consequences?

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## Recommendations (2011):

- 1) Sample should be put immediately in an **icy-water** slurry, and **plasma separated** from the cells within **30 minutes**.
- 2) If this cannot be obtained, a tube containing **rapid inhibitor** of glucose such as **citrate buffer** should be used.
- 3) Tubes with only **NaF** are **not enough** to prevent glycolysis

Sacks DB, Arnold M, Bakris GL, Bruns DE, Horvath AR, Kirkman MS, et al. Executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem* 2011;57:793-8.

# What is the clinical impact?

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- Implementing new citrate buffer tubes:
  - Higher mean glucose value for 0.8 mmol/L

*(Norman M, Jones I. Clin Biochem 2014;47:683-5.)*

- Higher prevalence of gestational diabetes

*(del Pino IG, et al. Clin Chem Lab Med 2013;51:1943-9.)*

- Higher prevalence of diabetes and lower prevalence of normal results

*(Juricic G, et al. Publication in process.)*



**10-18%**  
misdiagnosed patients?

# Guidelines and recommendations

- ❑ Existing guidelines use cut-off values based on the old tubes
- ❑ Revision of cut-off values is required using the new tubes
- ❑ Notify clinicians about the change


# 4. Hemolysis

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- Fluoride tubes have increased hemolysis rate
- NaF disrupts RBC membrane
- Catalase is released from RBC
- RBC glucose lower than serum


Clinical Biochemistry 46 (2013) 285–288

Contents lists available at SciVerse ScienceDirect

 **ELSEVIER**

Clinical Biochemistry

journal homepage: [www.elsevier.com/locate/clinbiochem](http://www.elsevier.com/locate/clinbiochem)



A comparison of glucose concentration in paired specimens collected in serum separator and fluoride/potassium oxalate blood collection tubes under survey 'field' conditions

Lois Fernandez <sup>a</sup>, Penny Jee <sup>a</sup>, Mari-jill Klein <sup>a</sup>, Peter Fischer <sup>a</sup>, Sherry L. Perkins <sup>b,c</sup>, Stephen P.J. Brooks <sup>a,\*</sup>

<sup>a</sup> Bureau of Nutritional Sciences, Food Directorate, Health Canada, Canada  
<sup>b</sup> Department of Pathology & Laboratory Medicine, The Ottawa Hospital, Ottawa, ON, Canada  
<sup>c</sup> Department of Pathology & Laboratory Medicine, University of Ottawa, Ottawa, ON, Canada

Hb > 0.15 g/L: **86.2%** NaF vs. **2.2%** SST

# Manufacturer declarations

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- Generally, glucose is not sensitive to hemolysis

Manufacturer	Hb conc. (g/L)	Glucose conc. (mmol/L)	Bias
<b>Abbott</b> (Abbott Park, IL, USA)	10 20	4.3 6,6	4.4% (10 g/L), 8.3% (20 g/L) 1.7% (10 g/L), 4.0% (20 g/L)
<b>Beckman Coulter</b> (Brea, California, USA)	5	?	<3%
<b>Roche</b> (Penzberg, Germany)	10	3.9	<10%
<b>AMS Diagnostics</b> (Weston, FL, USA)	1	?	<10%
<b>Pointe Scientific</b> (Canton, MI, USA)	?	?	? Do not analyse hemolysed samples.
<b>Teco Diagnostics</b> (Anaheim, CA, USA)	4	?	? Negligible interference.
<b>Thermo Scientific</b> (Waltham, MA, USA)	10	?	? No interference.

Acceptance criteria based on biological variation (Ricos et al. 2014):

$$I = 2.3\%, B = 1.8\%, TE = 5.5\%$$



# To conclude...

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Preamanalytical phase is the major source of variability for glucose measurement!

